New Claim 24 has basis at page 4, lines 6-10.

New Claims 25 and 26 have basis at page 4, lines 11-15.

New Claim 27 has support in original Claim 1. The additional phrase ". . . utilizing PEP as a precursor or intermediate" has support at page 3, lines 32-33.

New Claim 28 has support at page 4, lines 3-5.

New Claim 29 has support in original Claims 3-5.

New Claim 30 has support in original Claim 6.

New Claim 31 has support in original Claim 7.

New Claim 32 has support in original Claim 13. The additional phrase " . . . utilizing PEP as a precursor or intermediate" has support at page 3, lines 32-33.

New Claims 33, 34, 36 and 37 have support in original Claims 15, 16, 20 and 21, respectively.

New Claims 38 and 39 have support in original Claim 17.

Objection/Rejection Under 35 USC §112

The objection to the specification and rejection of Claims 1-22 are respectfully traversed. The Examiner contends that applicants have failed to adequately teach a method for increasing carbon flow from *any* carbon source into *any* metabolic pathway in *any* host cell.

Initially, applicants note that the claims as submitted herewith are limited to those metabolic pathways utilizing PEP as a precursor or intermediate and those host cells capable of utilizing a phosphotransferase transport system for carbohydrate transport. With respect to whether applicants have enabled the use of any carbon source, applicants point to the review article of Postma (Reference 6 cited in the specification, a copy of which is attached for the Examiner's

convenience). In Table 2 of Postma, the various carbohydrates transported by systems analogous to the glucose PTS system are listed. One can see that many sugars (i.e., glucose, sucrose, fructose, lactose, etc.) are transported by this system and, therefore, one skilled in the art would expect the presently claimed approach to work for any PTS sugar.

Also, at page 2, third paragraph, applicants describe the need for utilizing various carbon sources, including, for example, glucose, lactose, galactose, etc., for purposes of efficient biosynthetic production of desired compounds. Thus, applicants aver that while the present invention has been exemplified with glucose+ mutants, similar results could readily be achieved using other PTS sugars as the carbon source.

Furthermore, applicants contend that the present teachings should not be limited to *E. coli* since it is well documented (in the specification at pages 8-9 and in Postma submitted herewith) that the PTS system for all bacteria studied are composed of essentially the same protein components. The exact configuration of the proteins varies, but they accomplish the same task, i.e., transporting a PTS carbon source at the expense of PEP. Thus, given the disclosure in the specification as to suitable host organisms (based on their utilization of a PTS transport mechanism) and the known similarity of these systems from organism to organisms, applicants contend that this aspect of the §112 rejection is overcome.

Further support for applicants' position in this regard is found in the Saier reference cited by the Examiner. Saier et al. describe inactivating the *ptsH* and *ptsI* genes to yield PTS-*Salmonella typhimurium* host cells. Thus, the methods employed by applicants are useful in various organisms not just *E. coli*.

The Examiner further contends that increasing PEP may not increase carbon flow to the product because the first committed enzyme step of the pathway is not amplified. While it is often true that the first step in a biosynthetic pathway is generally well regulated, it is not necessarily true that this step is *rate limiting*. Furthermore, the present invention teaches increasing PEP availability by eliminating PEP-consuming processes (Pts-), then selecting a mutant suitable for fermentation processes that uses glucose as the carbon source (glu+). These mutants do not consume PEP during glucose transport, providing an opportunity for it to be used in another pathway.

Whether the first committed step in a pathway is rate-limiting depends largely on the availability of its substrates. For example, there are many examples of organisms (e.g., *Pseudomonas* bacteria) that lack transcriptional regulation of most biosynthetic pathways. In such organisms, carbon flow through these pathways is governed only by allosteric regulation (i.e., feedback inhibition). Since, in many cases, feedback inhibition is competitive or mixed-type in nature, increasing the substrate will indeed overcome inhibition and lead to increased carbon flow through the first pathway step (even without amplification). Therefore, increasing precursor availability to a pathway can, in fact, increase carbon flow through that pathway, even without amplification of the first enzymatic step. Accordingly, this aspect of the §112 rejection is believed to be overcome.

An additional argument put forth by the Examiner in the §112 rejection is the alleged unpredictability of combining PTS mutants with other carbon enhancement approaches such as *ppc, pyk,* and *pps*. The Examiner's claim of unpredictability with combined approaches is speculative at best. In fact, applicants have shown in work published after the filing date of this application that the effects of PTS and *pyk* are synergistic. (Gosset et al. (1996) J. Industrial Microbiology **17**:47-52 (enclosed).)

The final aspect of the §112 rejection is based on the Examiner's contention that applicants have only described one method for producing a phenotypically Pts- cell. While applicants have demonstrated one reproducible method for making Pts- cells, it is well within the state of the art to employ different methods to isolate Pts- cells. For example, it is well know that the antibiotic fosfomycin is transported through the PTS and that the selection of fosfomycin-resistant mutants is a method to isolate for internal and extended deletions of the PTS genes (see page 14 of the specification and Reference 28 cited therein). Such an approach has been used by several groups to isolate Pts- mutants without prior knowledge of the PTS genes. Furthermore, in the Postma review article, *supra*, several strategies for selecting Pts- mutants are discussed.

With respect to the Examiner's contention that the PTS genes have only been cloned from a limited number of organisms, applicants again point to Postma, *supra*. From Table 2 of this reference it is clear that the PTS genes from a wide variety of gram-positive and gram-negative bacteria have been cloned, and encode proteins having the same function.

Therefore, in view of the amendments and remarks provided herewith, it is respectfully submitted that the §112 objection to the specification and the rejection of Claims 1-22 have been overcome and should be withdrawn.

Rejection Under 35 USC §102(b)

Old Claims 1 and 2 (new Claims 27 and 28) and 17-19 (38 and 39) are rejected under 35 USC §102(b) as being anticipated by Biville et al. or Saier et al. This rejection is respectfully traversed.

Biville et al. teaches the isolation of mutants of *E. coli* producing pyrroloquinoline quinone. While Biville et al. started with strains having the PTS genes deleted, their mutants are quite different from applicants' because the prior art strains assimilated glucose by converting it (extracellularly) into gluconate, which is, in turn, presumably catabolized via the Entner-Doudoroff pathway. Thus, from one mol of glucose, this pathway produced one mol of glyceraldehyde-3-phosphate and 1 mol of pyruvate. Assuming that the glyceraldehyde-3-phosphate is further metabolized through the glycolytic pathway, it could produce 1 mol of PEP. Based on this finding, the PEP yield from the pathway of the prior art PTS- strains is the same as that obtained from a functional PTS strain, meaning that the PEP availability has *not* been increased. This is contrary to the mutants of the present invention which produce 2 mol of PEP for 1 mol of glucose.

Furthermore, the mutants of Biville et al. do not require galactose permease activity for glucose transport, while the mutants of the present invention require this activity to transport glucose.

Saier et al. describe the characterization of galactose permease mutants in *Salmonella typhimurium.* Two mutants were isolated in this work. The two parental strains used were deleted for the *ptsH* and *ptsI* genes only (the *crr* gene was left intact). The mutants were selected by their ability to grow on solid media. No attempts were made to isolate fast growing strains as with the present invention. In fact, the growth rate of the Saier et al. mutants is less than 0.4h and these mutants would have been eliminated by the continuous culture employed in Example 2 of the present invention.

It is well known that anticipation is a narrow and technical attack on patentability whose standards are strict. For example, the invention must be disclosed within the four corners of a single reference and this single reference cannot be modified by knowledge of those skilled in the art for purposes of anticipation. Thus, there is no anticipation unless all of the same elements are found in exactly the same situation and united in the same way to perform the identical function. Warner-Jenkinson Co. v. Allied Chemical Corp., 206 USPQ 837 (DC, SD NY 1979); Pfizer, Inc. v. International Rectifier Corp., 207 USPQ 397 (DC, CD CA, 1980).

New Claims 27 and 38 require selecting a host cell characterized by *requiring galactose permease activity to transport glucose* and *having a specific growth rate on glucose as a sole carbon source of at least about* $0.4h^{-1}$. These mutants are isolated for the purpose of increasing PEP availability to enhance overall carbon flow in a targeted metabolic pathway of the host cell. Neither of the cited references teaches each and every aspect of the selected host cells of the present claims. Given the strict standards for anticipation, it is readily apparent that there is no anticipation as described in Biville or Saier .

Rejection Under 35 USC §103

Old Claims 3, 5-16 and 20-22 are rejected under 35 USC §103 as being unpatentable over the combined disclosures of Frost, Holms and Biville or Saier. This rejection is respectfully traversed.

Frost teaches the overexpression of transketolase increases E4P availability. The reference is silent on the desire or need to increase PEP availability either alone (as an alternative strategy to *tkt* overexpression) or in combination with such.

Biville et al. teach the isolation of mutants of *E. coli* producing pyrroloquinoline quinone. As noted above, the mutants of Biville et al. are quite different from applicants' because they assimilated glucose by converting it (extracellularly) into gluconate, which is, in turn, presumably catabolized via the Entner-Doudoroff pathway. Thus, the PEP yield from the Biville mutants is *the same as* the one obtained from a functional PTS, *meaning that the PEP availability has not been increased.* This is contrary to the mutants of the present invention

which produce 2 mol of PEP for 1 mol of glucose and thereby enhance the availability of this substrate for the desired pathway.

Saier et al. taught certain Pts-/glucose+ strains, however, there is no mention in the reference of combining this approach with any other approach, such as amplification of *tkt*, to enhance carbon flow into the pathway.

Holms merely teaches what is readily admitted by applicants, that is, the PTS system is a major consumer of PEP. However, the reference is silent as to inactivating this system in conjunction with other carbon enhancing approaches as a means for enhancing carbon flow into the pathway.

The Examiner considers that the teachings of Frost showing one approach (overexpression of *tkt*) to increasing the amount of a substrate (E4P) for the first step in the common aromatic pathway, "would suggest to the ordinary skilled artisan the amplification of the other necessary precursor (PEP)" in order to enhance the availability of PEP. However, the teachings of Frost are silent as to this alternative approach to enhancing carbon flow and, therefore, provide no motivation to so combine the two methods. To the contrary, one could argue that the results of Frost (with *tkt*) were so convincing that the skilled artisan would not look beyond the effects of overexpressing transketolase.

Similarly, the teachings of Biville and Saier which deal with PTS- mutants provide no motivation to combine the claimed approaches, as these references are silent on alternative approaches such as amplification of tkt to enhance carbon flow.

The teachings of Holms add no further motivation to combine the approaches then did Frost, Biville or Saier. None of the cited references either alone or in combination teach or suggest the invention of the rejected claims. Furthermore, absent the teaching of the present invention, there is clearly no likelihood of success provided by any of these references. To the contrary, if the skilled artisan followed the Examiner's earlier contention[1] that the combination of multiple mutations to increase the flux of PEP or other substrates into a pathway such as the common

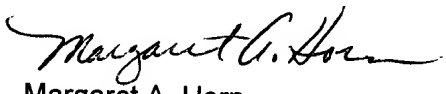
[1] See page 3 of Office Action.

aromatic pathway is highly unpredictable, no reasonable likelihood of success would be provided by the teachings of the cited references.

Therefore, applicants contend that the prior art cited by the Examiner provides no motivation to combine carbon enhancing approaches as recited in the rejected claims. As such, the obviousness rejection is believed to be overcome and should be withdrawn.

In view of the proposed amendments and remarks, it is believed that this application is in condition for allowance. Such action by the Examiner is earnestly solicited.

Respectfully submitted,



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